Impact of Tryptophan Oxidation on Native Fluorescence Detection

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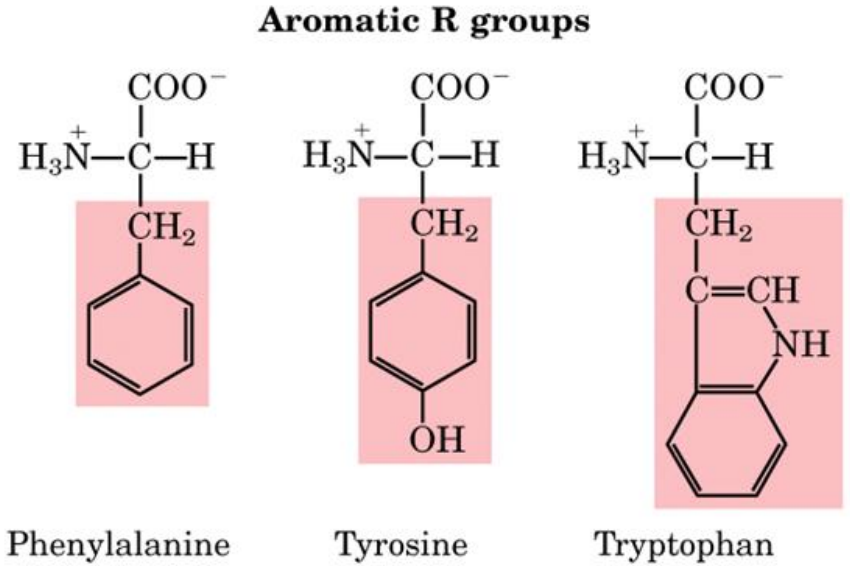
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Introduction

Native Fluorescence Detection

- In 2025, SCIEX released the BioPhase 8800 system equipped with native fluorescence (NF) detection
- NF employs a molecule's native fluorescence capabilities from aromatic amino acids
- NF eliminates baseline noise/interference that can interfere in UV spectra and does not require any additional labeling that is typically used in LIF methods



Early Evaluation Key Takeaways

- Of 14 tested Teva molecules, all peaks detected using UV detection are also detectable using NF detection
- Comparable results to traditional UV methods obtained when using NF
- Lack of baseline interference provides:
  - Confidence in identification of new peaks
  - Significant analyst and reviewer time savings
  - Ability to use automatic integration
  - Improved integration consistency

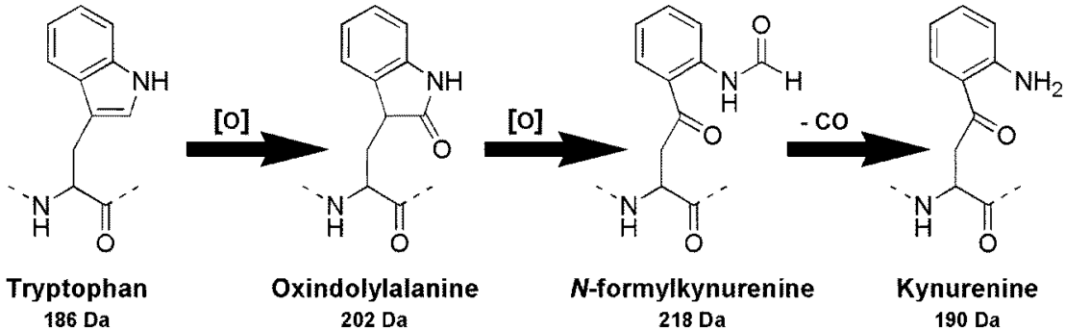
If NF signal is coming from tryptophan residues, how will changes to these residues impact CGE samples run using NF?



Experimental Plan



- The most common method of tryptophan modification is via oxidation



- Teva's Forced Degradation team developed a unique bench preparation method for chemical mAb tryptophan oxidation
  - Sample preparation induces tryptophan oxidation while not inducing other undesired degradation pathways

Sample	Total Oxidation (%)*
Control	0.7
Oxidation Exposed	43.9

*Determined via Peptide Mapping

***Oxidation isolated to HC**

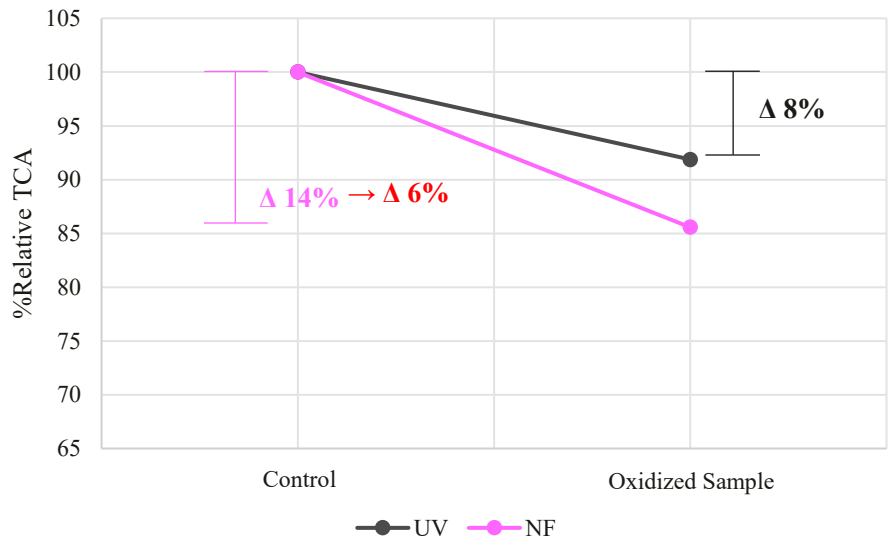
- Samples were run under non-reduced and reduced conditions using UV and NF detection

Results- Total TCA

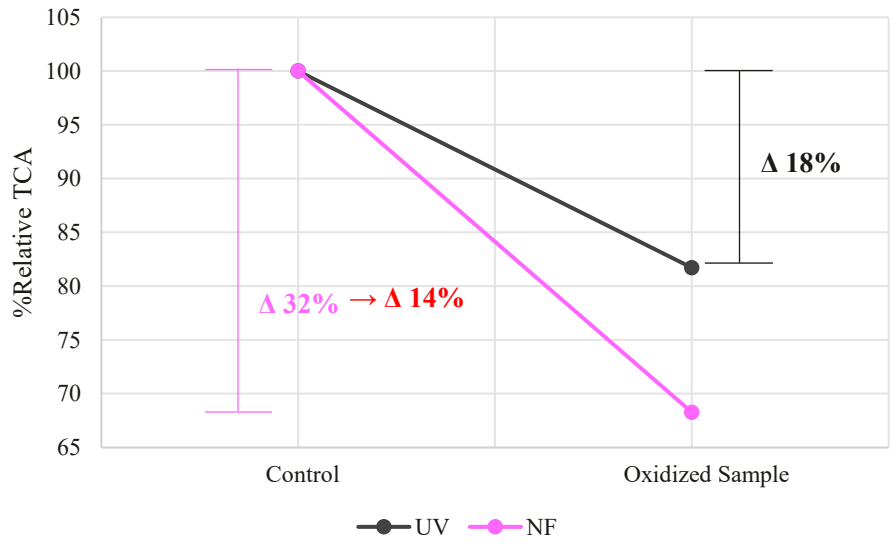
UV samples also showed a loss of signal due to the sample stress

!! **MUST** normalize NF results against the UV results to isolate the impact of oxidation on NF from changes caused by the stress itself

Reduced



Non-Reduced

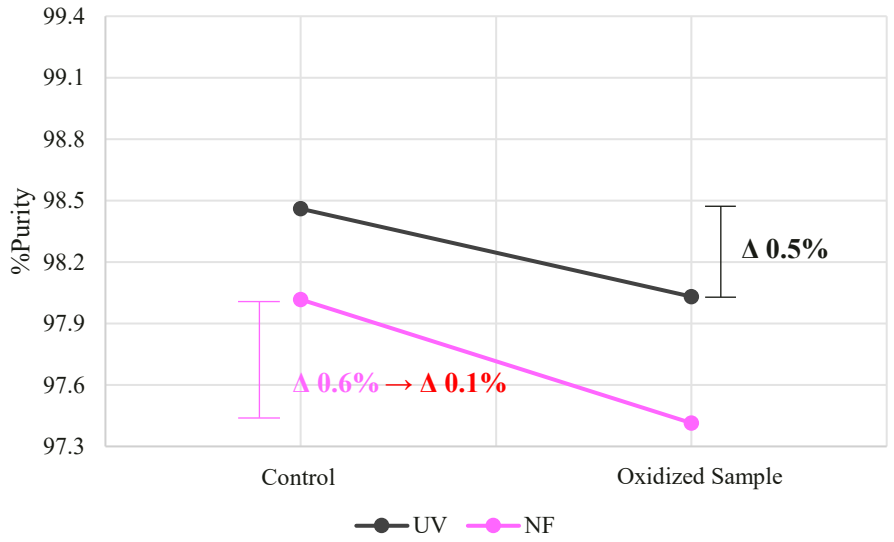


Results- %Purity

Samples run on both detectors showed a loss in %Purity due to sample oxidation

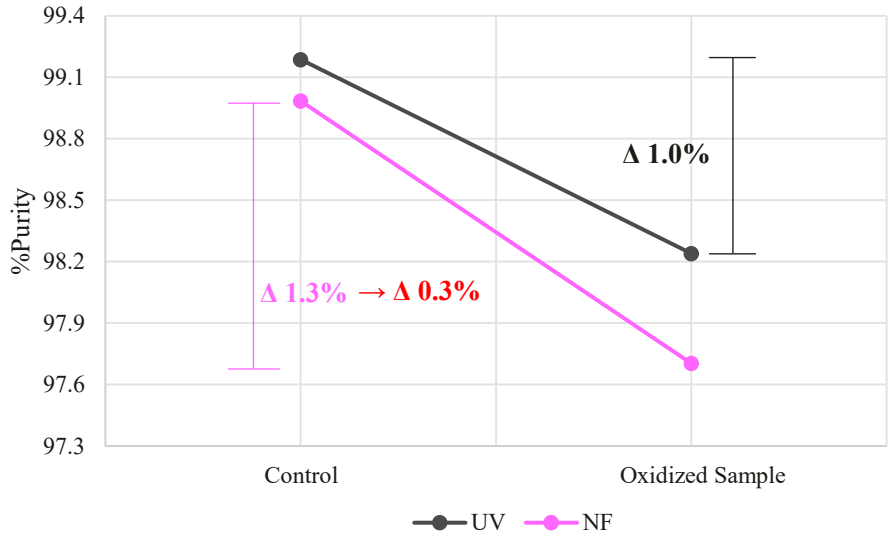
!! **MUST** normalize NF results against the UV results to isolate the impact of oxidation on NF from changes caused by the stress itself

Reduced



%Purity = %LC + %HC

Non-Reduced

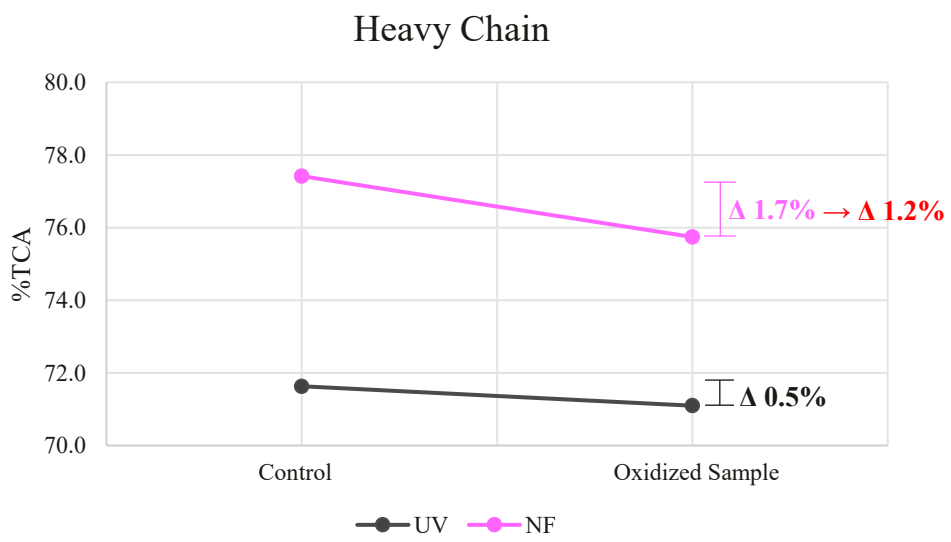


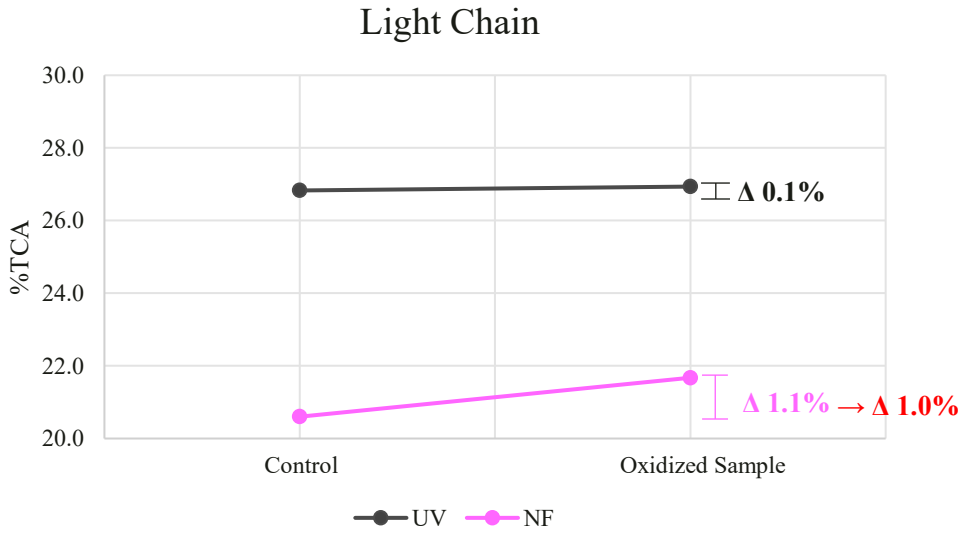
%Purity = %Intact IgG

*%Purity differences observed for NF vs UV is due to integration differences (e.g. continuous baseline in NF, detection of additional impurity species in NF, etc.)

Case Study Take Aways

- While exaggerated signal loss was observed when using NF detection, the impact to reported results was minimal
- It is important to also assess the individual peak data
  - As part of the early evaluation, it was noted that some molecules show an increase in HC:LC using NF due to the differences in the number of tryptophans in the LC and HC between molecules





- The number of tryptophans per chain does result in differences in individual peak results for this molecule

Overall Take Aways



- Tryptophan oxidation does not significantly impact the utility of NF detection for the molecule studied here
- It is possible that the signal loss caused by tryptophan oxidation could be conflated with the impact of the degradation itself
- Molecule-by-molecule screenings may need to be performed before routinely implementing NF detection
  - To ensure all peaks present in UV are detectable using NF
    - Thus far, all peaks present in UV have been detectable using NF
  - To identify a molecule's susceptibility to tryptophan oxidation and its impact on CGE analysis

- Additional follow up study was completed to assess assay Range due to the higher signal intensity of NF
  - The NF detector has a lower linear range allowing for the inclusion of lower concentration samples, decreased impact of salt interference, and improved detection of closely migrating species

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Thank
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